



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12Q 1/68</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/36573</b> <b>(43) International Publication Date:</b> 22 July 1999 (22.07.99)
<b>(21) International Application Number:</b> PCT/US99/01017 <b>(22) International Filing Date:</b> 19 January 1999 (19.01.99) <b>(30) Priority Data:</b> 09/009,107              20 January 1998 (20.01.98)              US <b>(71)(72) Applicants and Inventors:</b> SCHICHMAN, Steven, A. [US/US]; 17 Marble Court, Little Rock, AR 72212 (US). PARKINSON, Bruce [US/US]; 600 Breakwater Drive, Fort Collins, CO 80525 (US). <b>(74) Agent:</b> ELMAN, Gerry, J.; Elman & Associates, 20 West Third Street, P.O. Box 1969, Media, PA 19063-8969 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> DETECTION OF GENETIC INFORMATION		
<b>(57) Abstract</b>  A semiconductor detector device for detecting DNA hybridization is described. The device provides a detection system comprising a semiconductor substrate which forms a platform on which hybridization may be performed, and the site of attachment of specific single-stranded DNA molecules attached thereto. The device detects electrons which are conducted from chemical labels through double-stranded DNA formed between complementary single-stranded probe nucleotides and target polynucleotides. Electron-acceptor and electron-donor embodiments are described. Also described are electron-transfer chemical labels that are attached to single-stranded DNA molecules.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece			<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>ML</b>	Mali	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MN</b>	Mongolia	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MR</b>	Mauritania	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MW</b>	Malawi	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>MX</b>	Mexico	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NE</b>	Niger	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NL</b>	Netherlands	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NO</b>	Norway	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>NZ</b>	New Zealand		
<b>CM</b>	Cameroon			<b>PL</b>	Poland		
<b>CN</b>	China	<b>KR</b>	Republic of Korea	<b>PT</b>	Portugal		
<b>CU</b>	Cuba	<b>KZ</b>	Kazakstan	<b>RO</b>	Romania		
<b>CZ</b>	Czech Republic	<b>LC</b>	Saint Lucia	<b>RU</b>	Russian Federation		
<b>DE</b>	Germany	<b>LI</b>	Liechtenstein	<b>SD</b>	Sudan		
<b>DK</b>	Denmark	<b>LK</b>	Sri Lanka	<b>SE</b>	Sweden		
<b>EE</b>	Estonia	<b>LR</b>	Liberia	<b>SG</b>	Singapore		

## DETECTION OF GENETIC INFORMATION

### Technical Field

The invention provides for the detection of genetic information, e.g., for use within the fields of molecular genetics, molecular oncology, and molecular infectious disease, to provide for the detection of genetic deficiencies and abnormalities and the detection of patterns of gene expression.

### Background Art

Methods to detect specific gene sequences within the human genome are central to the field of human molecular genetics. One method for gene analysis is the direct determination of the actual deoxyribonucleic acid ("DNA") nucleotide sequence using manual or automated DNA sequencing technology. Indirect methods for the detection of gene sequences usually involve the use of nucleic acid probes which specifically bind to target sequences in selected genes. Nucleotide probes bind to target sequences through a process known as hybridization.

Hybridization refers to the noncovalent interaction through hydrogen bonding between two single-stranded nucleic acid molecules. Hydrogen bonding occurs between so-called complementary nucleotide bases. For the double-stranded DNA molecule, the nucleotide "base" adenine ("A") is complementary to the base thymidine ("T"); these bases form so-called A:T base pairs. Similarly, the base guanosine ("G") is complementary to the base cytosine ("C"), forming G:C base pairs. For example, an oligonucleotide with the sequence 5'-ATTTGGCATCAT-3' is perfectly complementary to the oligonucleotide 3'-TAAACCGTAGTA-5', where 5' and 3' refer to the orientation of the 5' and 3' hydroxyl groups of the deoxyribose/phosphate backbone of the linear oligonucleotide. These two complementary single-stranded oligonucleotides will hybridize with one another to form a double-stranded DNA molecule when they are mixed together under proper conditions.

The strands of a double-stranded DNA molecule will dissociate from one another when heated above a temperature that is referred to as the "melting temperature" or "melting point," ("T<sub>m</sub>"). The T<sub>m</sub> of an oligonucleotide in aqueous solution is determined by the ionic strength and pH of the solution, and by the length and base composition of the oligonucleotide. The T<sub>m</sub> increases with the length of the oligonucleotide. The T<sub>m</sub> also increases with the percentage of G+C base composition.

In a typical hybridization reaction, a probe and a target DNA are mixed together in a buffered aqueous or non-aqueous solution, heated to a temperature above the T<sub>m</sub>, and then

slowly cooled to a temperature below the  $T_m$ . As the temperature is slowly lowered, complementary sequences "anneal" to one another as their  $T_m$  is reached. For oligonucleotides of known length and base composition, conditions of temperature and ionic strength can be chosen that permit discrimination between perfectly complementary oligonucleotides and those with  
5 bases that are non-complementary at one or more positions. For many years, oligonucleotide hybridization has been used to detect single-base mismatches in gene sequences (so-called allele-specific oligonucleotide hybridization).

Specific genes may differ from their normal or consensus sequences for several reasons.  
10 First, natural alleles occur, such as the hemoglobin allele that gives rise to sickle cell disease. Such alleles are maintained in the population by selective pressures. In the case of the sickle cell gene, it is believed that the gene confers a certain resistance to malaria. Second, nucleotide changes arise by mutation. Mutations are believed to be responsible for the development of certain cancers, and mutations that result in the production of defective proteins are known to  
15 cause diseases such as hemophilia.

Gene "expression" involves the transcription of ribonucleic acid ("RNA") molecules from a DNA "template." RNA differs from DNA in that the sugar moiety is ribose instead of deoxyribose, and the base uridine ("U") occurs in place of thymidine.  
20

Nucleic acid probes are labeled so that a detection scheme can be employed to determine the presence or absence of a target sequence to which a probe is complementary. Detection schemes currently in use employ either radioactive or non-radioactive labels. Emissions from radioactive labels, such as  $^{32}\text{P}$ ,  $^{33}\text{P}$ , and  $^{35}\text{S}$ , are detected with x-ray film or phosphor image  
25 analyzers. Non-radioactive detection schemes utilize fluorescent or chemiluminescent labels. The magnitude of a signal produced by a bound probe is often increased by forming further complexes such as streptavidin/biotin complexes, or digoxigenin/anti-digoxigenin complexes. Different detection schemes are suitable for different applications.

The first widely used system to detect specific gene sequences was the Southern blot, developed in the mid-1970's by Edwin Southern. The Southern blot is a detection system in which target DNA, immobilized on a solid support, is hybridized with a labeled nucleic acid probe. Target DNA is first cleaved into pieces by the use of restriction enzymes that cleave the DNA at  
30 the location of certain specific nucleotide sequences. The DNA fragments are then separated according to size by electrophoresis through a gel matrix. The separated DNA fragments are then denatured and transferred from the gel to a solid support, typically nitrocellulose or nylon filter  
35

membranes. The size of the blot is determined by the size of the electrophoresis gel. Commonly used gels are 20 cm x 20 cm, and so-called "minigels" are as small as 5 cm x 5 cm.

5 In the Southern process the DNA is transferred, or "blotted," by capillary action. The DNA is then covalently linked to the solid support, usually by heat treatment or ultraviolet irradiation. Target DNA bound to a membrane is placed in contact with a liquid containing labeled probes, and any probe that is not specifically bound to the target DNA is removed by washing the blot. The location of the bound probe on the membrane is revealed with a detection system appropriate for the type of label used. The label may be radioactive or non-radioactive.  
10 The Southern blot, still in wide use today, is one of the fundamental techniques of molecular genetics.

A variation on the Southern blot is the so-called "dot blot." A dot blot consists of target DNA (or RNA) as a pattern of spots or dots directly fixed on a solid support membrane made of nitrocellulose or nylon. Dot blot systems do not use a gel and do not use electrophoresis to separate DNA fragments by size. Thus, the name "dot blot" is somewhat of a misnomer, because the DNA is not blotted onto the membrane from a gel. The target nucleic acid on a dot blot may be DNA or RNA, and it may be genomic DNA, recombinant DNA cloned in a vector, specific segments of DNA amplified by the polymerase chain reaction ("PCR") or another *in vitro* amplification technique, or any other kind of hybridizable nucleic acid molecule.  
15  
20

Typically, dot blots are prepared with target DNA from various different sources, e.g., from different people or from different recombinant clones. Dots of target DNA are arrayed in rows and columns on a single piece of membrane; this arrangement is called a "DNA array." The spots or dots of nucleic acid in a DNA array of this sort are usually about 0.5 cm in diameter, and the filter array may contain about 100 samples on a membrane that measures approximately 12 cm x 15 cm. Sometimes dot blots are arrayed in a single row on a strip of membrane filter. Dot blots are hybridized in a manner similar to Southern blots with probes labeled radioactively or non-radioactively. Dot blots typically yield qualitative data with regard to the presence or absence of the target nucleic acid for which the probe is complementary. It is possible, however, to quantify the signals from dot blots if the signal varies linearly with the amount of target DNA in each sample. Allele-specific oligonucleotide hybridization (see above) is usually done in a dot blot format. Thus, one example of the use of dot blots is to examine genomic DNA from many different people for the presence of a point mutation in a specific target gene (i.e. many different samples are arrayed on one filter and examined with a single labeled probe).  
25  
30  
35

The term "reverse dot blot" refers to a system in which the oligonucleotide probes are attached to a membrane filter. Different probes are typically arrayed in rows and columns on a

single-membrane filter. Sometimes different probes are arrayed in a single row on a filter strip. The oligonucleotide probes, which are not labeled, are covalently attached to the membrane by heat, UV treatment, or chemical bonding. In a reverse dot blot system, the probe array on the filter is hybridized with labeled target DNA isolated from a single source, such as genomic DNA from one person. Because the labeled entity is the target DNA rather than the probe DNA, this system is the "reverse" of the dot blot system described above. A reverse dot blot may be used, for example, to examine the DNA from one person for many different mutations. In such a test system, probes for a series of different mutations are arrayed on one filter, and labeled DNA from an individual is in the hybridization fluid applied to the filter. The dimensions and array density of a reverse dot blot are typically the same as a standard dot blot.

During the past six years, technologies have been developed to miniaturize the reverse dot blot system to a microscopic scale. The goal of these technologies is the development of a clinical laboratory instrument for genetic testing, cancer testing, and testing for infectious disease organisms. Efforts have been directed toward decreasing the size of the hybridization array to 1 cm x 1 cm or less while increasing the "array density" (i.e., the number of points in the array) to greater than 1000. Microminiaturization is advantageous because it increases the number of probes that can be tested simultaneously and decreases the volume of sample DNA and reagents consumed per hybridization reaction. The most commonly used material for the hybridization platform is silicon, but glass and other types of materials are also used. In the most commonly used system, arrays of oligonucleotide probes are synthesized directly onto the surface of a silicon chip.

Microarrays with oligonucleotide probes synthesized directly onto the surface are often referred to as "DNA biochips." Localized oligonucleotide synthesis is accomplished in part through the use of masking techniques derived from the microelectronics industry or chemjet technology derived from the ink-jet printer industry. Hybridization of microarrays with labeled target DNA is done in a small volume of liquid on the surface of the DNA biochip. Biochips may also be enclosed in a small flow chamber that is accessed and managed by microfluidic engineering.

Fluorescence-, chemiluminescence-, and radioactivity-based detection systems are adaptable to biochip arrays. However, at present only fluorescence-based detection systems are in common use. Typically, a fluorescent labeled DNA is used in currently employed systems in conjunction with a fluorescence detector that scans the surface of the chip. Chemistries for labeling DNA with fluorescent tags are well developed and commercially available. Sensitive, high resolution fluorescence scanning detectors have been developed, but these devices are bulky, very expensive and relatively slow. It is reported that chemiluminescence detectors for biochips are

under development, but their performance characteristics are presently unknown. Radioactivity-based detection systems present health hazards and regulatory problems because of the potentially harmful emissions from the isotopes used. These systems also are more difficult to use, especially in a clinical laboratory setting due to the short half-lives of most isotopes. Thus, there is a need for a new detection system that is applicable to DNA biochip technology and that is fast, inexpensive, sensitive, and capable of very high resolution.

#### Disclosure of Invention

The invention disclosed herein provides a semiconductor detector device used to detect DNA hybridization. One embodiment of the novel semiconductor detection system of the present invention comprises a semiconductor substrate with specific single-stranded DNA molecules attached thereto. Attached to each single-stranded DNA molecule is one or more chemical labels that are either electron-donors or electron-acceptors, as described with more particularity below. Complementary single-stranded target polynucleotides hybridize to the specific single-stranded DNA molecules to form double-stranded DNA. The semiconductor substrate allows the detection of electrons which are conducted through the double-stranded DNA.

Another embodiment of the novel semiconductor detection system of the present invention comprises a semiconductor substrate with specific single-stranded DNA molecules attached thereto. In this embodiment of the present invention, one or more chemical labels that are either electron-donors or electron-acceptors are attached to second single-stranded DNA molecules, as described with more particularity below. Complementary single-stranded target polynucleotides hybridize to the attached single-stranded molecule and the probe-bearing single-stranded molecule to form double-stranded DNA. The semiconductor substrate allows the detection of electrons which are conducted through double-stranded DNA.

In certain embodiments of the present invention, the novel semiconductor detection system detects electrons which are conducted to the substrate through double-stranded DNA from an electron donor. In other embodiments of the invention, electrons are conducted from the substrate to chemical labels that are electron acceptors.

In the present invention an "anchor-substrate" is a single structural semiconductor substrate that performs three functions. First, it functions as the substrate to which an "anchor-probe" is attached; second, it forms the hybridization platform, and third, it acts as part of the detector. In the present invention, a single-stranded DNA "electron-transfer probe" is complementary to a target DNA and may be an anchor-probe. In an embodiment of the present invention where the single-stranded DNA probe is an anchor-probe, such a probe is attached to an

anchor-substrate at its 3' end and has an electron-donor or an electron-acceptor moiety attached at its 5' end. Also, in the presence of a hybridizable target DNA under hybridizing conditions, double-stranded DNA is formed, and detectable electron flow occurs to or from the anchor-substrate.

5

The present invention uses semiconductor chip material as one element in an electron transfer system. The semiconductor material forms part of the detector device. Either p-type or n-type semiconductors may be used in embodiments of the present invention. In a device of the present invention that uses an n-type semiconductor, electron transfer occurs from an electron-donor group on the specific DNA strand into the conduction band of the semiconductor (so-called "electron injection"). In a device of the present invention that uses a p-type semiconductor, electron transfer occurs from the valence band of the semiconductor into an electron-acceptor group on the specific DNA strand (so called "hole injection").

10

15

In one embodiment of the present invention, the anchor-probe is an electron-transfer probe that is directly coupled to the anchor-substrate. The electron-transfer probe bears one or more chemical labels which are electron-donor or electron-acceptor groups. In this embodiment of the invention, whenever double-stranded DNA is formed by hybridization, electrons may be conducted from the chemical label to the anchor-substrate. Double-stranded DNA is formed by hybridization of a single-stranded target nucleotide and the oligonucleotide anchor-probe.

20

The present invention also provides novel electron-transfer probes, namely probes that have electron-transfer moieties coupled to them. Such electron-transfer moieties are electron-donor moieties or electron-acceptor moieties. In an "electron-donor" embodiment of the invention, the electron-transfer probe, when excited, generates electrons. The electrons are then conducted through the double-stranded DNA that is formed by hybridization. Such electrons are detectable by the semiconductor material that serves as a detector device. In an "electron-acceptor" embodiment of the invention, the electron-transfer probe, when excited, accepts electrons. The electrons are then conducted away from the substrate through the double-stranded DNA that is formed by hybridization, and the current thus established is detectable through the semiconductor material that serves as a detector device.

25

30

In another embodiment of the present invention, an "anchor-probe" is directly coupled to the anchor-substrate to form an anchor-probe-substrate. The probe on such an anchor-probe-substrate does not bear a chemical moiety. In this embodiment of the invention an electron-transfer probe bears a chemical moiety that is an electron-donor or an electron-acceptor, and the anchor-probe and the electron-transfer probe are contiguously complementary to a target

35



nucleotide. Double-stranded DNA is formed by hybridization of the anchor-probe, the electron-transfer probe and the target nucleotide. In an "electron-donor" embodiment of the invention, the chemical labels of the electron-transfer probe, when excited, generate electrons which are then conducted through the double-stranded DNA to the semiconductor material that serves as a detector device. In an "electron-acceptor" embodiment of the invention, the chemical labels of the electron-transfer probe, when excited, can accept electrons. Electrons are conducted through the double-stranded DNA from the semiconductor material.

A further embodiment of the present invention is an electron-transfer probe composition including a single-stranded nucleic acid and one or multiple electron-transfer moieties in which at least one of the electron-transfer moieties is a transition-metal complex, where the transition metal is a member of the group Cadmium, Magnesium, Copper, Cobalt, Osmium, Iridium, Platinum, Palladium, Zinc, Iron or Ruthenium. In one form of this embodiment of the invention, the single-stranded nucleic acid is attached to an electrically responsive anchor-substrate, and the transition-metal complexes are covalently attached to a sugar of the sugar-phosphate backbone of a nucleic acid. The nucleic acid in certain embodiments is RNA and in other embodiments of the present invention is DNA. In yet a further embodiment of the present invention, an electrode is an electron-transfer moiety. Electron-transfer moieties of the present invention are attached to nucleic acids at the 3' position of deoxyribose, when the nucleic acid is DNA, and at either the 2' or 3' position of ribose when the nucleic acid is RNA.

Yet a further embodiment of the present invention is an electron-transfer probe composition including a single-stranded nucleic acid and one or multiple electron-transfer moieties in which at least one of the electron-transfer moieties is a dye, where the dye is an organic dyes such as those from the rhodamine, coumarin, methine or thionin families of dyes. Such dyes are excited by using a monochromatic light source such as a laser, or a filtered or unfiltered polychromatic light source. Suitable dyes for used in the present invention are cresyl violet, methylene blue and ruthenium trisbipyridine.

One aspect of the present invention is a composition-of-matter comprising: a first hybridizable single-stranded oligonucleotide electron-transfer probe, and a second hybridizable single-stranded oligonucleotide anchor-probe. The electron-transfer probe is complementary to a first portion of a target nucleotide sequence, and the anchor-probe is complementary to a second portion of a target nucleotide sequence. In this embodiment of the invention, the first portion of the target nucleotide sequence and the second portion of the target nucleotide sequence are contiguous to one another. In other words, the two sequences are complementary to adjacent sequences on the target nucleotide strand which have no bases between them. In this

embodiment the probe of the anchor-probe-substrate has an electron-transfer group coupled to it, and the substrate is a semiconductor that forms part of the electronic detection system.

5 The aforementioned embodiment of the present invention can be targeted to any known gene sequence and used in a process to qualitatively analyze gene sequences or to quantitatively analyze gene expression. This process, also part of the present invention, permits detection of specific mutations present in genes associated with different types of cancer and permits detection of mutations in genes known to be associated with different types of genetic disease.

10 The process of the present invention is also used to detect genes in heterozygous individuals that are "carriers," i.e. those who are not affected by the mutation they carry. The process also can also be used to detect specific patterns of gene expression in normal and abnormal tissues, including neoplastic disease tissue.

15 The process of the present invention may also be used to detect gene sequences derived from micro-organisms (e.g. bacteria, fungi, and viruses) that may cause infectious diseases in humans and/or other animals. The process can also be used to screen combinatorial libraries

#### Brief Description of the Drawings

20 Figure 1 is a diagram of the process of a first embodiment of the present invention.

Figure 2 is a diagram of the process of a second embodiment of the present invention.

Figure 3(a) illustrates an embodiment of the present invention with an electron-donor chemical moiety and figure 3(b) shows an embodiment of the present invention with an electron-acceptor chemical moiety.

25 Figure 4 is a diagram of a device of the present invention showing double-stranded nucleic acid molecules on the surface of a semiconductor substrate.

Figure 5 is a diagram of a multi-segmented device of the present invention.

#### Modes for Carrying Out the Invention

30 The invention disclosed herein provides a novel semiconductor detector device to detect DNA hybridization that is a unitary structural semiconductor substrate, i.e., an "anchor-substrate," that functions (1) as the surface to which an anchor-probe is attached, (2) as a hybridization platform, and (3) as a detector. The novel detection system detects the electrons which are conducted through double-stranded DNA from electron-generating chemical labels or to electron-accepting labels. Double-stranded DNA is formed by DNA hybridization with oligonucleotide probes that bear chemical "electron-transfer" labels that can be excited by  
35 light to generate or accept electrons. Electrons conducted through the DNA activate an "anchor-substrate" semiconductor material that is a novel detector device.

The present invention also provides a process that utilizes a semiconductor detector to detect electrons transferred through DNA from an electron-generating label, or to an electron-accepting label. Photographic grains may also be used as detectors. Electrons are conducted very easily along double-stranded DNA, whereas electrons conducted poorly along single-stranded DNA. Therefore, the transfer of electrons from an excited  
5 electron-generating label to the substrate, measured as current flow per interval of time, or by reduction of a photographic grain, can be used to detect the presence of double-stranded DNA or to discriminate between double-stranded DNA and single-stranded DNA molecules attached to the surface of a detector of the present invention. A potential difference maintained between the solution and the anchor-substrate may additionally motivate the flow of electrons to or from a label.

10 Devices of the present invention are formed in an embodiment such as a matrix, or grid, comprising numerous different DNA anchor-probes. Such an embodiment of the present invention enables simultaneously testing for multiple target DNAs. Results from such a matrix embodiment of the present invention can be read-out from the device by scanning the device with a focussed light beam or by electrical multiplexing, for  
15 example, by using individually patterned diodes or transistors which correspond to each pixel.

A particular embodiment of the present invention comprises an array of different single-stranded DNA oligomer molecules arranged in a grid on the semiconductor surface. Arrays are envisaged that be a single field,  
20 or up to 1000 x 1000 pixels, or even more. Each pixel of a grid of the present invention bears an oligonucleotide that is characteristic of a specific gene, gene mutation, or microorganism.

A preferred embodiment of the present invention has a substrate that is a semiconductor material selected from the following: amorphous silicon ( $\alpha$ -Si), the III-IV semiconductor class, such as gallium arsenide, gallium phosphide, gallium nitride or indium phosphide; the II-VI semiconductor class such as cadmium  
25 sulfide, zinc selenide or zinc sulfide; the I-VII materials such as silver chloride, silver bromide or silver iodide, or the oxide semiconductors such as titanium dioxide, strontium titanate or zinc oxide. Other substrates suitable for use in the present invention will be known to those of skill in the art.

The semiconductor substrate may be monocrystalline, polycrystalline, amorphous or nanometer-sized  
30 semiconductor particles formed into a thin film of semiconducting material. The semiconductor materials may be of the doped n-type for detection of photoexcited electrons injected into the conduction band of the semiconductor. Embodiments of the present invention that are devices which detect electrons transferred from electron-donor groups, use n-type semiconductors as the anchor-substrate. P-type semiconductors are used in  
35 embodiments of the present invention for the detection of photoexcited holes injected into the valence band. Embodiments of the present invention that are devices which detect electrons transferred to electron-acceptor groups, use p-type semiconductors as the anchor-substrate. Semiconductors used for anchor-substrates in

embodiments of the present invention may be also undoped, as would be the case for nanoscale semiconductor particle films.

5 An anchor-probe of the present invention is a single-stranded DNA molecule that is complementary to a target nucleotide sequence of interest. An anchor-probe complementary to any target nucleotide of interest may be used in the present invention. Anchor-probes are bound to the surface of the semiconductor by chemical forces such as a covalent bond, or by electrostatic binding, or by hydrophobic interactions. Examples of covalent bonds include, but are not limited to, alkyl thiol bonds, amine condensation links, phosphate, silane, thiol, ester, amide or carboxylate links or links formed by free radical reactions with alkenes. Other means of linking DNA to solid substrates will be known to those of skill in the art. Linkages may be formed directly or 10 by means of bifunctional linker molecules between the 3' hydroxyl group of the anchor-probe DNA and the anchor-substrate. An extensive discussion of the labelling and modification of nucleic acids may be found in U.S. Patent 5,591,578, the disclosure of which is hereby incorporated by reference.

15 The nucleotide sequence of the anchor-probe is complementary to a portion of the specific gene sequence to be detected. In preferred embodiments of the invention, anchor-probes range from 14 to 30 nucleotides in length. Longer or shorter oligomer lengths are also functional in the present invention as will be known to those of skill in the art. In preferred embodiments of arrays of the present invention, a different anchor-probe is used for each pixel.

20 In a preferred embodiment of the present invention, the presence of a specific segment of target nucleic acid is signaled by electrons that are conducted through double-stranded DNA which is formed by the process of the invention. In one embodiment of the present invention, the double-stranded DNA is assembled on the surface of the semiconductor detector device by the hybridization of two complementary nucleotide sequences. 25 The two pieces are: (1) an anchor-probe which is an electron-transfer probe that comprises a DNA segment to which the chemical label that transfers electrons is attached, and (2) DNA derived from the sample to be tested.

30 In another embodiment of the present invention, the double-stranded DNA is assembled on the surface of the semiconductor detector device by the hybridization of three complementary nucleotide sequences. The three pieces are: (1) an anchor-probe that comprises a DNA segment that is attached, or anchored, to the detector surface; (2) DNA derived from the sample to be tested; and (3) an electron-transfer probe that comprises a DNA segment to which the chemical label that transfers electrons is attached.

35 Electron-transfer probes are single-stranded DNA oligonucleotides, typically less than 15 nucleotides in length. The sequence of an electron-transfer probe is complementary to the sequence of the sample DNA to be tested. The sequence of the electron-transfer probe is selected to be complementary to a sequence in the target DNA that is contiguous to the sequence that is complementary to the anchor-probe.

Electron-transfer probes of the present invention are populations of single-stranded DNA molecules with electron-transfer groups attached at the 5' end. Among the electron-transfer groups that are used in embodiments of the present invention are organic dyes such as those from the rhodamine, coumarin, methine or thionin families of dyes. However, the present invention is not limited to these dyes. Other suitable dyes will be known to those of skill in the art. The electron-transfer group has a function similar to that of a photographic sensitizer and could also be from the class of dyes that includes cyanine dyes. Other suitable electron-transfer groups are metal complexes such as ruthenium trisbipyridine or other chromophoric metal complexes.

Anchor-probes may also be synthesized directly on the surface of the anchor-substrate by techniques that are well known to those of skill in the art.

To perform the assay of the present invention, the hybridization process is first performed, and one then detects binding of the target DNA sequences by directing a photon excitation source, such as a laser, a polychromatic light source, or a filtered polychromatic source, at the surface array. Dyes and corresponding wavelengths of light suitable for used in the present invention are: cresyl violet with 633 nm light from a helium-neon laser, methylene blue and 680 nm light from a red diode laser, and ruthenium trisbipyridine and 574.5 nm light from an argon laser.

The wavelength of the light source is selected so that photoexcitation of the chemical label of the electron-transfer probe is achieved. The wavelength of light used in the present invention to excite the chemical label is selected to be of an energy that does not directly excite the semiconductor. In certain embodiments of the present invention, the intensity of the light is modulated at a convenient frequency to allow for phase detection of the signal. This technique selectively increases the detectability of DNA-specific photo-initiated electron-transfer, above any background dark-electron-transfer processes.

Transfer of an electron from the electron-transfer group to the anchor-substrate oxidizes the electron-transfer group. Therefore, in another embodiment of the invention, a reducing agent is added to the solution covering the array in order to reduce photooxidized electron-transfer probe molecules so that they can be repeatedly photooxidized. This technique causes each attached double-stranded DNA molecule to provide many electrons for detection. Addition of a reducing agent to the solution used in the system chemically reduces the oxidized electron-transfer group, thereby altering the state of the group so that it may again be activated by the exciting light, thus enabling another cycle of electron transfer.

One can accomplish detection of binding of target DNA to an array of the present invention in at least two ways. In one such process, the excitation light is scanned sequentially over the array, and detection of a photocurrent is accomplished with a single electrical contact to the back of the array. With this embodiment of a detection system, the location of pixels to which electron-transfer probes have hybridized, is determined from

the position of the scanning excitation beam. In an alternate detection process, the entire array is bathed in light and individual grid points on the array are electrically addressed via multiplexing electronics. The signal-to-noise ratio with either method can be increased by integrating the photocurrent over time to accumulate photocurrent signal.

5

The hybridization process of the present invention involves the interaction of either two or three single-stranded DNA molecules. These molecules have sequences which hybridize to form a contiguous double-stranded DNA only when the molecules are complementary to one another.

The process comprises the following steps:

10

(1) the test DNA is incubated under hybridizing conditions on an anchor-probe-substrate of the present invention that bears an electron-transfer moiety. Test DNA that contains a nucleotide sequence complementary to the probe hybridizes and becomes bound;

(2) unhybridized test DNA is washed away;

15

(3) the substrate is irradiated with light to photoexcite the electron-transfer moiety, and the signal generated by the photoexcitation is electronically detected as described above.

Another process of the present invention to detect the presence of a target DNA comprises the following steps:

20

(1) the test DNA and an electron-transfer probe are incubated under hybridizing conditions on an anchor-probe-substrate of the present invention that does not bear an electron-transfer moiety. In this embodiment of the process, test DNA that contains a nucleotide sequence contiguously-complementary to the electron-transfer probe and the anchor-probe hybridizes and becomes bound;

(2) unhybridized test DNA and unhybridized electron-transfer probe are washed away;

25

(3) the formed complexes are treated with DNA ligase to ligate adjacent oligonucleotides. In another embodiment of this process, the ligation step is omitted;

(4) the substrate is irradiated with light to photoexcite the electron-transfer group on the electron-transfer probe, and the signal generated by the photoexcitation is electronically detected.

30

The sample DNA to be tested could be from any source, such as a cell line, the cells of a human, or any other organism. In a preferred embodiment, specific regions of the sample DNA are amplified, e.g., by a technique such as PCR. For the qualitative detection of gene defects, either linear or exponential amplification is used. The amount of amplification required is that needed to achieve a good signal-to-noise ratio by the detector.

35

In another embodiment of the present invention, detection of gene expression may be achieved. To detect gene expression, RNA is obtained from a source such as a cell line or the cells of a human or other animal. The RNA is first converted into complementary DNA ("cDNA"). The cDNA can be used without

amplification as the sample in an assay of the present invention. Alternatively, for RNAs expressed at low levels, a specific region of the cDNA can be further amplified, e.g., by a linear or exponential amplification technique, so as to achieve a concentration that will yield a detectable signal.

5           Another invention that is disclosed herein is a composition-of-matter with two components, namely the electron-transfer probe and the anchor-probe. The electron-transfer probe is complementary to a first target nucleotide sequence, and the anchor-probe is complementary to a second target nucleotide sequence that is contiguous with, and adjacent to, the first target nucleotide sequence. In this embodiment of the present invention the electron-transfer probe has an electron-transfer group coupled to it, and the anchor-probe is  
10           coupled to a semiconductor that forms part of an electronic detection system.

          The process of detecting a specific DNA sequence with the present invention may comprise a step wise hybridization process in which three pieces of single-stranded DNA are assembled to form double-stranded DNA anchored to a semiconductor at one end and containing an electron-transfer group at the other end. A step  
15           wise process is carried out in four steps.

          Step 1 consists of hybridizing a sample DNA on an anchor-probe-substrate of the present invention. Sample DNA that is complementary to the anchor-probe will hybridize with the anchor-probe on the anchor-probe-substrate and the anchor-substrate is then washed to remove unbound sample DNA.  
20

          Step 2 consists of hybridizing an electron-transfer probe to the complex formed in step 1 on the anchor-probe-substrate. In the preferred embodiment of the present invention, a mixture of electron-transfer probes is used, with each individual probe specific for an individual anchor-probe DNA. In another embodiment of the invention, a single electron-transfer probe is used, such a probe being complementary to a  
25           segment of DNA added onto the sample DNA. Electron-transfer probes will hybridize with complementary sample DNA sequences that are, in turn, hybridized to the anchor-probes attached to the anchor-substrate by the previously described physical or chemical means. Electron-transfer probe sequences are designed so as to be contiguous with the anchor-probe sequences.

          Step 3 consists of a ligation step catalyzed by a DNA ligase enzyme. Specific hybridization of the anchor-probe and electron-transfer probe with a complementary sample DNA will precisely align the 5' end of the anchor-probe DNA with the 3' end of the electron-transfer probe DNA such that the ends can be ligated together to form a single contiguous molecule. For example, the DNA ends may be ligated together with T4  
30           DNA Ligase, provided an adjacent 5'-phosphate and a 3'-hydroxyl are present.  
35

Step 4 consists of a washing step performed with an aqueous solution of salt and detergent under conditions that will wash off electron-transfer probes that are not ligated to anchor-probes. Because the unligated probes are short sequences, the washing will be quantitative.

5 Ligation of the adjacent ends is not necessary for efficient conduction of electrons through the double-stranded DNA. However, ligation of adjacent anchor-probe and electron-transfer probe oligonucleotides permits more stringent washing to be used in step 4. Also, if the sample DNA contains a mismatch at or very close to the adjacent ends of the anchor-probe and the electron-transfer probe, then ligation of the anchor-probe and electron-transfer probe will be inhibited. Thus, the ligation step enables discrimination between normal and mutant sequences.

10 Another embodiment of the process uses conventional silver halide emulsions as the substrate, with the hybridized DNA segments acting as the sensitizer of the silver halide grains, as is routinely accomplished with the similar organic dyes in conventional photography. In this embodiment of the invention, the results of the assay are ascertained by developing the film sheet and examining or scanning the film for exposed regions indicating a positive DNA match.

Embodiments of the present invention may be understood by reference to the drawings. Figure 1 diagrammatically illustrates the process of the simplest embodiment of the present invention. Figure 1(a) shows a single-stranded DNA anchor-probe 101 with a terminally attached electron-donor chemical label 102 attached to anchor-substrate 103. Figure 1(b) shows a single-stranded target nucleotide 104 adjacent to the DNA anchor-probe 101 attached to anchor-substrate 103 with terminally attached electron-donor chemical label 102 attached to DNA anchor-probe 101. Figure 1(c) shows single-stranded target nucleotide 104 hybridized to the single-stranded DNA anchor-probe 101. Arrow 105 in figure 1(c) indicates the direction of the flow of electrons from the terminally attached electron-donor chemical label 102 to the anchor-substrate 103 through the double-stranded DNA structure 111 upon excitation of electron-donor label 102 by exciting light 110.

Figure 2 diagrammatically illustrates the process of an embodiment of the present invention. Figure 2(a) shows an unlabeled single-stranded DNA anchor-probe 201 attached to anchor-substrate 203 with a single-stranded target nucleotide 204 adjacent thereto. Figure 2(b) shows the single-stranded target nucleotide 204 hybridized to the unlabeled single-stranded DNA anchor-probe 201 which is attached to anchor-substrate 203. Figure 2(c) shows a hybridizable single-stranded electron-transfer probe 206 with a terminally attached chemical electron-donor label 202 adjacent to the single-stranded DNA anchor-probe 201 with the single-stranded target nucleotide 204 hybridized thereto. Figure 2(d) shows the single-stranded electron-transfer probe 206 with a terminally attached electron-donor chemical label 202 hybridized to the single-stranded DNA single-stranded target nucleotide 204. The gap 207 between the single-stranded electron-transfer probe 206 with a terminally attached electron-donor chemical label 202 and the anchor-probe 201 illustrates that these two probes



are not ligated together to covalently join them. Arrow 205 in figure 2(d) indicates the direction of the flow of electrons from the electron-donor chemical label 202 to the anchor-substrate 203 through the double-stranded DNA structure 211 upon excitation of electron-donor label 202 by exciting light 210.

5           Figure 3 shows two embodiments of the present invention. Figure 3(a) shows a single-stranded target nucleotide 304 hybridized to a single-stranded DNA anchor-probe 301 with an electron-donor probe 302 attached thereto. Arrow 305 in figure 3(a) indicates the direction of the flow of electrons from the terminally attached electron-donor chemical label 302 to the anchor-substrate 303 through the double-stranded DNA structure 311 upon excitation of electron-donor chemical label 302 by exciting light 310. Figure 3(b) shows a  
10          single-stranded target nucleotide 304 hybridized to a single-stranded DNA anchor-probe 301 with an electron-acceptor probe 308 attached thereto. Arrow 309 in figure 3(b) indicates the direction of the flow of electrons to the terminally attached electron-acceptor chemical label 308 from the anchor-substrate 303 through the double-stranded DNA structure 311 upon excitation of electron-acceptor chemical label 308 by exciting light 310.

15           Figure 4 is a diagram of a device of the present invention that shows double-stranded nucleic acid molecules 411 assembled on the surface of semiconductor substrate 412. Upon excitation of terminally attached electron-donor chemical label 402 with exciting light 410, electrons are conducted to the semiconductor anchor-substrate 412 through the double-stranded nucleotide structure 411 upon excitation of electron-donor label 402 by exciting light 410. An electronic detection system 413 reads out the signal from  
20          semiconductor anchor-substrate 412.

            Figure 5 is a diagram of a multi-segmented device of the present invention showing different double-stranded nucleic acid molecules, (DNA 1, DNA 2, DNA 3, etc.,) assembled on the surface of semiconductor substrate 512. Upon excitation of terminally attached electron-donor chemical labels electrons are conducted to  
25          the semiconductor anchor-substrate 512 through formed double-stranded nucleotides and electronic detection system 513 reads out signals from the semiconductor anchor-substrate 512 through a series of connectors 514.

            The detailed description and examples presented above are for purposes of illustration. Other embodiments which employ the principles of the invention and fall within the spirit and scope thereof will be  
30          apparent from the present disclosure to those skilled in the art.

**CLAIMS**

What is claimed is:

1. A method for detecting a target nucleic acid sequence, said method comprising:
  - b) attaching a first nucleic acid probe to an electrically receptive anchor-substrate wherein said first nucleic acid probe is complementary to a first portion of a target nucleic acid sequence;
  - a) attaching one or more electron-donor moieties to a second nucleic acid probe wherein said second nucleic acid probe is complementary to a second portion of said target nucleic acid sequence and wherein said first portion and said second portion are contiguous nucleic acid sequences;
  - c) hybridizing a DNA sample and said second nucleic acid probe acid probe on said electrically receptive anchor-substrate with said first nucleic acid probe thereon to form a hybridized mixture;
  - d) washing said electrically receptive anchor-substrate to remove unbound material;
  - e) irradiating said electrically receptive anchor-substrate to excite said electron-donor moieties;
  - f) detecting a signal generated by electrons transferred from excited electron-donor moieties to said electrically receptive anchor-substrate.
2. A method according to claim 1 additionally comprising ligating said hybridized mixture after step c) and before to step d).
3. A method according to claim 1, wherein said electrically receptive anchor-substrate further comprises a detector.
4. A method according to claim 3, wherein said method is performed in a solution on said hybridization platform.
5. A method according to claim 1, wherein said electrically receptive anchor-substrate is selected from the group consisting of a nanocrystalline titanium dioxide film, a crystalline semiconductor material comprising a single crystal, a polycrystalline semiconductor material, an amorphous semiconductor material and a photographic emulsion.
6. A device comprising an anchor-substrate that is an electron-acceptor having attached thereto a first single-stranded nucleic acid.
7. The device of claim 6 wherein said first single-stranded nucleic acid has attached thereto one or more electron-donor moieties.

8. A device according to claim 6, wherein said anchor-substrate that is an electron-acceptor additionally comprises an electron detector and is adapted to form a hybridization platform.
9. A device according to claim 6, wherein said anchor-substrate is selected from the group consisting of a nanocrystalline titanium dioxide film, a crystalline semiconductor material comprising a single crystal, a polycrystalline semiconductor material, an amorphous semiconducting material and a photographic emulsion.
10. A composition comprising:  
a first single-stranded nucleic acid, having attached thereto one or more electron-donor moieties, and  
a second single-stranded nucleic acid attached to an anchor-substrate that is an electron acceptor;  
wherein said first single-stranded nucleic acid and said second single-stranded nucleic acid are contiguously-complementary to a target single-stranded nucleic acid, and  
said first single-stranded nucleic acid and said second single-stranded nucleic acid are adapted to form double-stranded DNA, upon hybridization to a homologous nucleic acid target sequence,  
whereby electron transfer occurs between said electron-donor and said anchor-substrate when said double-stranded DNA is formed.
11. A composition according to claim 10, wherein said anchor-substrate that is an electron acceptor further comprises an electron detector and is adapted to form a hybridization platform.
12. A composition according to claim 10, wherein said anchor-substrate is selected from the group consisting of a nanocrystalline titanium dioxide film, a crystalline semiconductor material comprising a single crystal, a polycrystalline semiconductor material, an amorphous semiconducting material and a photographic emulsion.
13. A electron-transfer probe composition comprising:  
a single-stranded nucleic acid and one or multiple electron-transfer moieties;  
wherein at least one of said electron-transfer moieties is a transition-metal complex, comprising a transition metal selected from the group consisting of Cd, Mg, Cu, Co, Os, Ir, Pt, Pd, Zn, Fe and Ru.
14. An electron-transfer probe composition of claim 13, additionally comprising:

an electrically responsive anchor-substrate with said single-stranded nucleic acid attached thereto, and wherein:

said electron-transfer moieties are covalently attached to sugar moieties of the sugar-phosphate backbone of said nucleic acid;  
whereby transfer of electrons can occur between said electron-transfer moieties and said electrically responsive anchor-substrate when a target nucleotide sequence is hybridized to said single-stranded nucleic acid.

15. An electron-transfer probe composition of claim 13, wherein:

one of said electron-transfer moieties is an electrode and at least one other of said electron-transfer moieties is a transition-metal complex;  
whereby electron transfer can occur between said transition-metal complex and said electrode when a target nucleotide sequence hybridizes to said single-stranded nucleic acid.

16. An electron-transfer probe composition of claim 13, wherein:

said single-stranded nucleic acid is DNA or RNA.

17. The electron-transfer probe composition of claim 16, wherein:

said single-stranded nucleic acid is DNA,

said sugar moiety is deoxyribose, and

said electron-transfer moieties are transition-metal complexes covalently attached to the 3' position of said deoxyribose.

18. The electron-transfer probe composition of claim 16, wherein:

said single-stranded nucleic acid is RNA,

said sugar moiety is ribose, and

said electron-transfer moieties are transition-metal complexes covalently attached to the 2' or 3' position of said ribose.

19. A electron-transfer probe composition comprising:

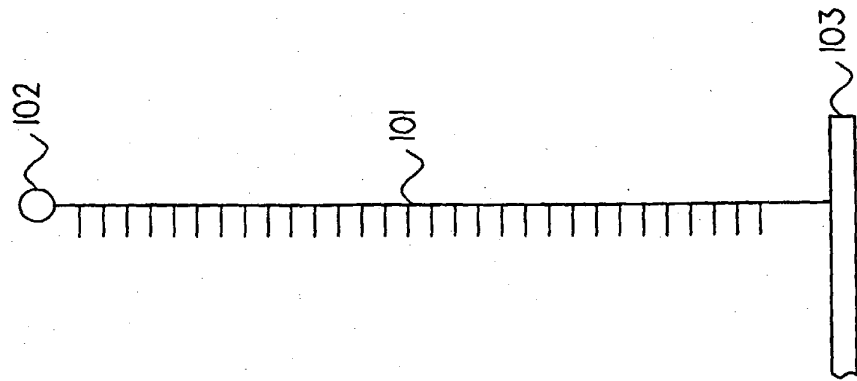
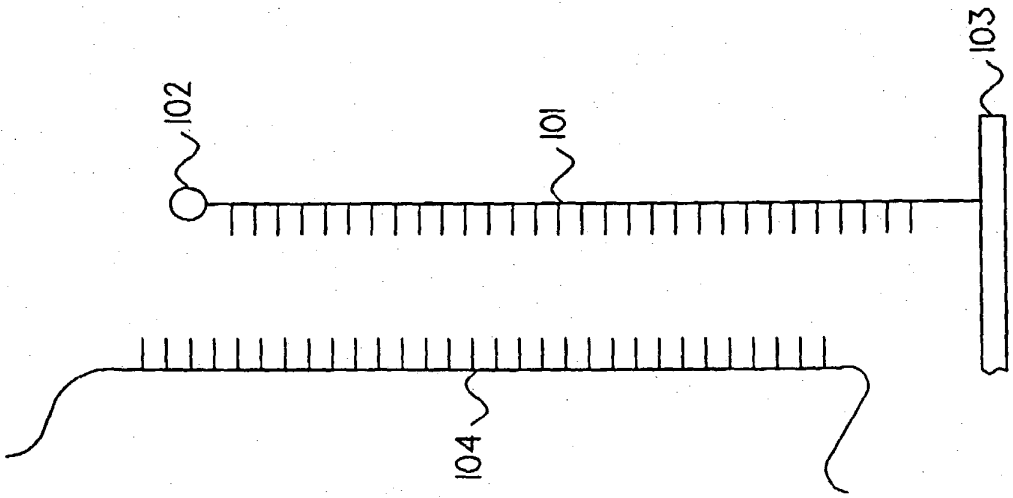
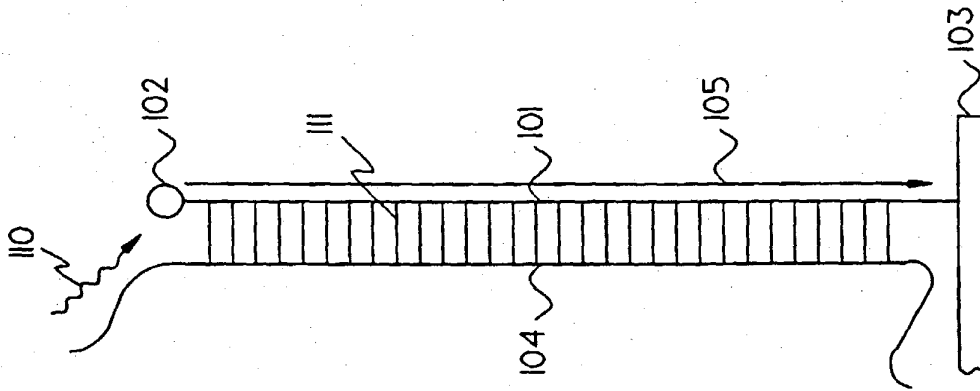
a single-stranded nucleic acid and one or multiple electron-transfer moieties;

wherein at least one of said electron-transfer moieties is a dye selected from the group consisting of cresyl violet, methylene blue and ruthenium trisbipyridine.

20. An electron-transfer probe composition of claim 13, additionally comprising:

an electrically responsive anchor-substrate with said single-stranded nucleic acid attached thereto, and wherein:

said electron transfer moieties are covalently attached to sugar moieties of the sugar-phosphate backbone of said nucleic acid;  
whereby transfer of electrons can occur between said electron-transfer moieties and said electrically responsive anchor-substrate when a target nucleotide sequence is hybridized to said single-stranded nucleic acid.



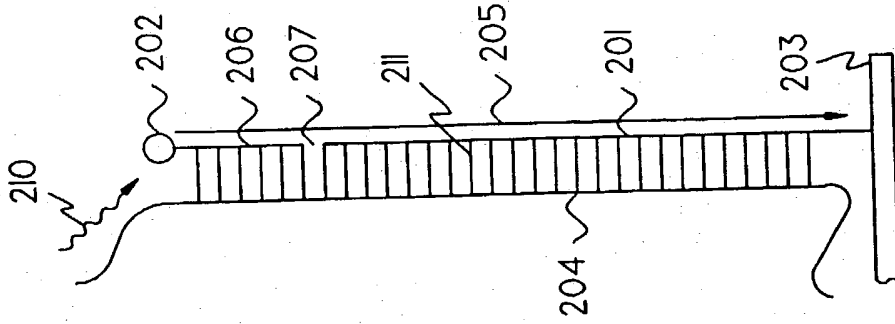


FIG-2D

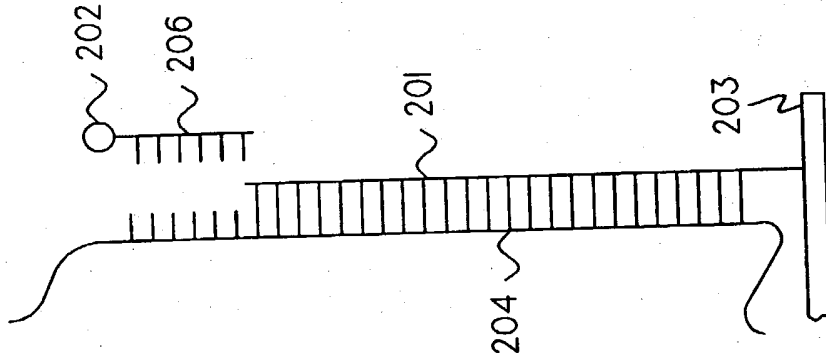


FIG-2C

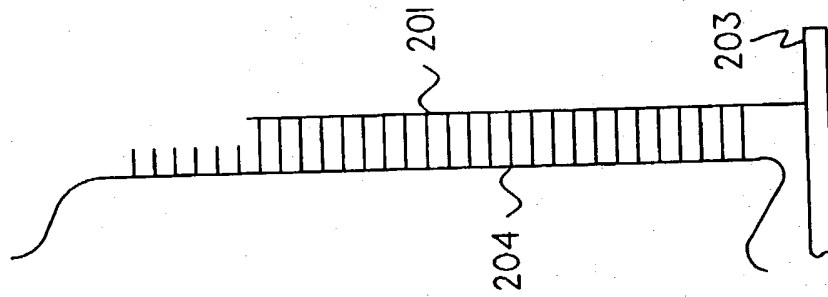


FIG-2B

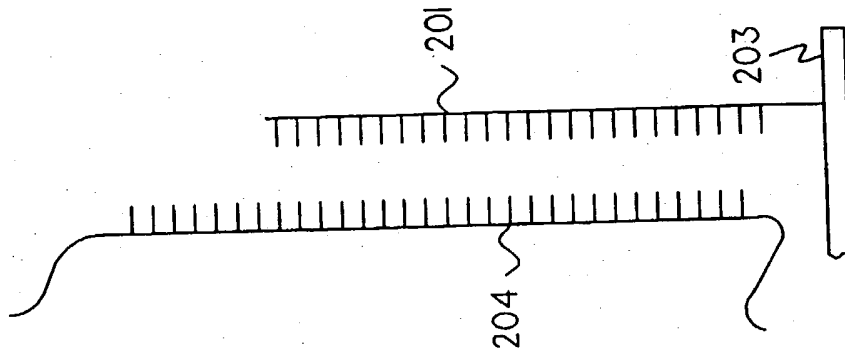


FIG-2A

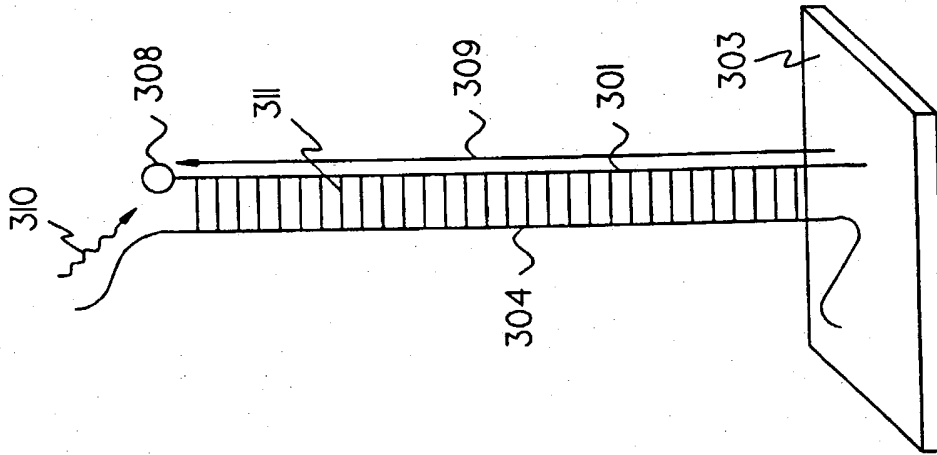


Fig-3B

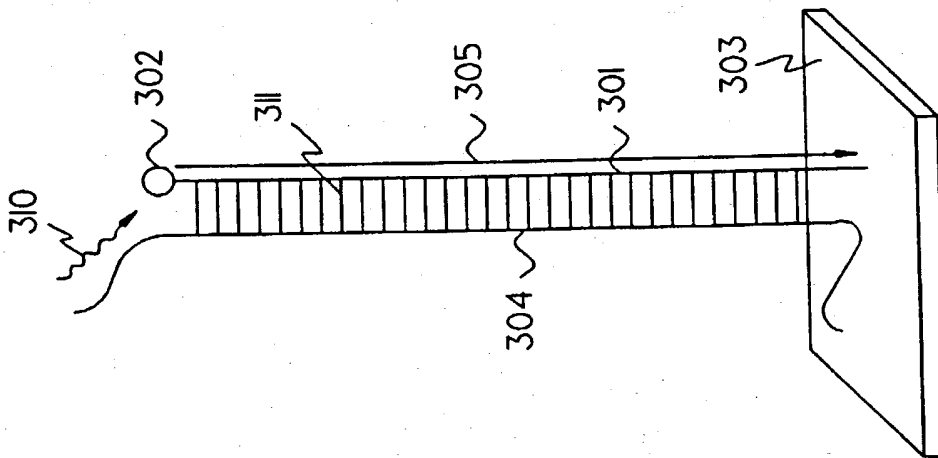


Fig-3A



4/4

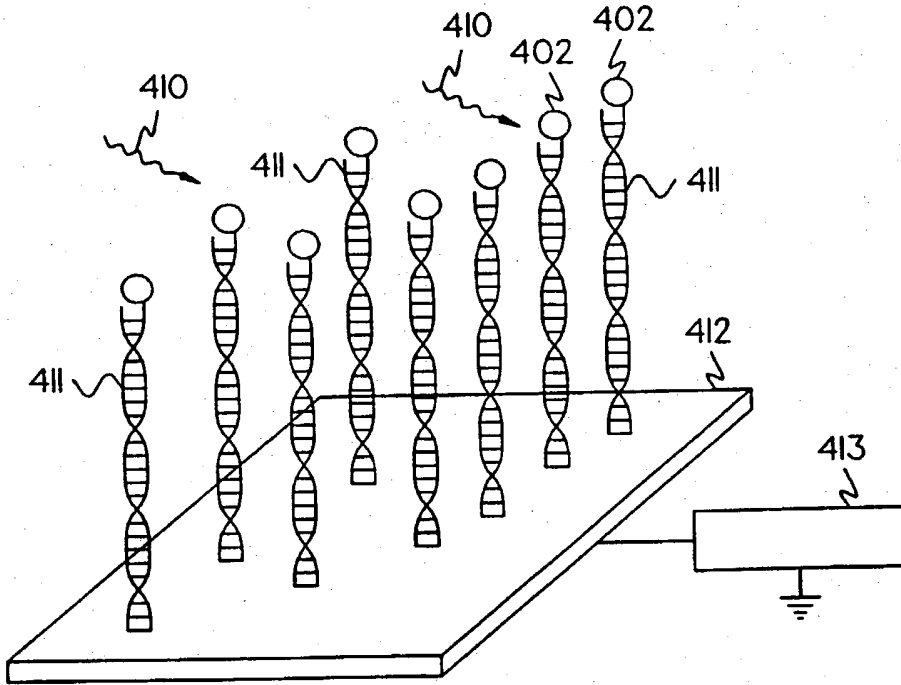


Fig-4

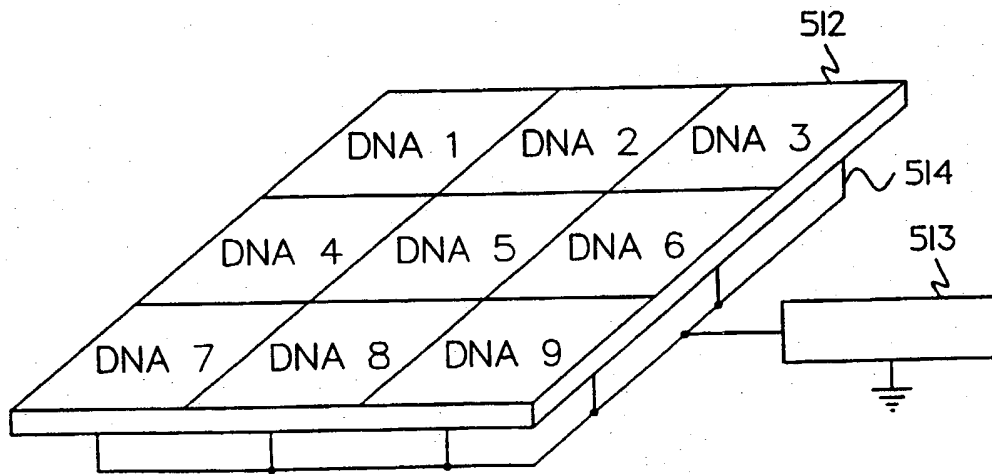


Fig-5

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/01017

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :C12Q 1/68  
US CL :435/6

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/50,68.1,69,82.01,82.02; 435/6,91.1,91.2,283.1,287.1,287.2; 536/22.1,23.1,25.3; 935/77,78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATERSON, D. Electric Genes. Scientific American. May 1995, pages 33-34, see entire disclosure.	1-20
A	FROMHERZ, P. et al. Photoinduced Electron Transfer in DNA Matrix from Intercalated Ethidium to Condensed Methyl- viologen. Journal of the American Chemical Society. 1986, Volume 108, pages 5361-5362, see entire disclosure.	1-20
A	KELLY, S. et al. Electrochemisty of Methylene Blue Bound to a DNA-Modified Electrode. Bioconjugate Chemistry, 1997, Volume 8, Number 1, pages 31-37, see entire disclosure.	1-20

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

26 MARCH 1999

Date of mailing of the international search report

22 APR 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

*D. Lawrence Fox*  
ARDIN MARSCHEL

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/01017

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, 5,472,881 A (BEEBE et al.) 05 December 1995, see the abstract and column 5, lines 13-41.	6
Y	US 4,840,893 A (HILL et al.) 20 June 1989, see entire disclosure.	1-20
Y	WO 97/01646 A2 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 16 January 1997, see claims 1-151 and Figures 3, 4, 7, and 8.	1-20
X - Y	US 5,591,578 A (MEADE et al.) 07 January 1997, see the entire disclosure and especially column 16, line 10, through column 17, line 28.	6-8 and 13-20 ----- 1-5 and 9-12

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US99/01017

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, MEDLINE, WPI, BIOTECH ABS, BIOSIS

search terms: conductive oligomer, current, electron donor, acceptor, electrode